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family with subclasses perhaps possessing different modes of regulation.

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Both the conserved and the unique gene structure of stomach-specific calpains reveal processes of calpain gene evolution.

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Hata S, Nishi K, Kawamoto T, Lee HJ, Kawahara H, Maeda T, Shintani Y. Sorimachi H, Suzuki K.

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan.

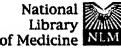
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The proteins nCL-2 and nCL-2' are members of the Ca2+-dependent cysteine protease (calpain) superfamily, with stomach-specific expression. Like other typical calpains, nCL-2 has three distinct domains, a protease, a C2-like, and a 5EF-hand Ca2+-binding domain, as well as the N-terminal propertide region. On the other hand, nCL-2' lacks the C2-like and 5EF-hand domains but is otherwise identical to nCL-2, except for the three C-terminal residues. To examine the stomach-specific and presumed alternative expression mechanisms of nCL-2 and nCL-2', we have cloned and characterized the mouse gene for nCL-2 and nCL-2'. The mouse nCL-2 gene contains at least 23 exons, spanning more than 50 kb, and possesses an exon specific for nCL-2' in the middle. Therefore, nCL-2 and nCL-2' are generated by alternative splicing of the same gene, Capn8. Capn8 shows the highly conserved gene organization of the other typical calpain large subunit genes, CAPN1, CAPN2, CAPN3, CAPN9, CAPN11, and Capn12, except for the unique exon between exon 9 and exon 10 of Capn8, which encodes the 3' half of the nCL-2' transcript. No such exon in th corresponding regions was found in CAPN1, CAPN2, CAPN3, CAPN9, or CAPN11. Gene and cDNA structures of a presumed human orthologue of mous nCL-2, CAPN8, were determined, revealing that it overlaps human CAPN2, the gene for the m-calpain large subunit, in head-to-head orientation at 1q32-41. These features of Capn8 and CAPN8 illustrate a process of calpain gene evolution, i.e., the protease, C2-like, and 5EF-hand domains presumably functioned as independent genes, and the calpain superfamily has evolved by ordered fusions of these ancestral gene units, with subsequent amplifications.

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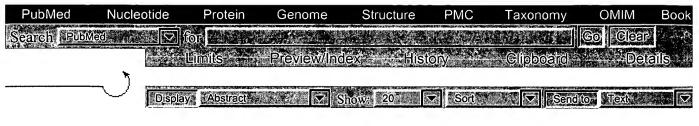
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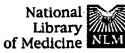
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US-PAT-NO: 6235481

DOCUMENT-IDENTIFIER: US 6235481 B1

TITLE: Polynucleotides encoding calpain 10

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

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Oda; Naohisa	Nagoya			JP
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Cox; Nancy J.	Inverness	IL		

US-CL-CURRENT: 435/6; 536/23.1, 536/24.1

CLAIMS:

What is claimed is:

- 1. An isolated and purified polynucleotide comprising a region encoding human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10.
- 2. The polynucleotide of claim 1, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
- 3. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:2.
- 4. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:4.
- 5. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:6.
- 6. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:8.
- 7. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:10.
- 8. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:12.
- 9. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:14.

- 10. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:16.
 - 11. The polynucleotide of claim 2, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:18.
- 12. The polynucleotide of claim 2; wherein the region has the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, or SEQ ID NO:19.
 - 13. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:1.}$
 - $14.\ \, {
 m The\ polynucleotide}$ of claim 12, wherein the region has the sequence of SEQ ID NO:3.
 - 15. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:5}$.
 - 16. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:7.}$
 - 17. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID NO:9.
 - 18. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:11}$.
 - 19. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:13}$.
 - 20. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:15}$.
 - 21. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID ${\tt NO:17}$.
 - 22. The polynucleotide of claim 12, wherein the region has the sequence of SEQ ID NO:19.
 - 23. A vector comprising a polynucleotide that encodes human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10.
 - 24. The vector of claim 23, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
 - 25. The vector of claim 24, wherein the region has the sequence of SEQ ED NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, or SEQ ID NO:19.
 - 26. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:1.
 - 27. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:3.
 - 28. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:5.
 - 29. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:7.
 - 30. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:9.
 - 31. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:11.
 - 32. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:13.

- 33. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:15.
- 34. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:17.
- 35. The vector of claim 25, wherein the region has the sequence of SEQ ID NO:19.
- 36. The vector of claim 23, further comprising a promoter.
- 37. The vector of claim 23, wherein the vector is a viral vector.
- 38. The vector of claim 23, wherein the vector is a retroviral vector.
- 39. The vector of claim 23, wherein the vector is a plasmid.
- 40. A recombinant host cell comprising a polynucleotide that encodes human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10.
- 41. The recombinant host cell of claim 40, further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
- 42. The recombinant host cell of claim 41, wherein the region has the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, or SEQ ID NO:19.
- 43. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:1.
- 44. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:3.
- 45. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:5.
- 46. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:7.
- 47. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:9.
- 48. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:11.
- 49. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:13.
- 50. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:15.
- 51. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:17.
- 52. The recombinant host cell of claim 42, wherein the region has the sequence of SEQ ID NO:19.
- 53. The recombinant host cell of claim 40 wherein the host cell is further defined as a prokaryotic cell.
- 54. The recombinant host cell of claim 40, wherein the host cell is a eukaryotic cell.

- 55. The recombinant host cell of claim 54, wherein the host cell is a mammalian cell.
- 56. The recombinant host cell of claim 55, wherein the host cell is a human cell.
- 57. A method of obtaining a human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10 polypeptide comprising:
- a) obtaining a polynucleotide comprising a region encoding a human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10;
- b) inserting the polynucleotide into a host cell; and
- c) culturing the host cell under conditions sufficient to allow production of the human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10 polypeptide;

wherein a human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10 polypeptide is thereby obtained.

- 58. The method of claim 57, further comprising the step of isolating the human calpain 10a, human calpain 10b, human calpain 10c, human calpain 10d, human calpain 10e, human calpain 10f, human calpain 10g, human calpain 10h, or mouse calpain 10 polypeptide from the host cell.
- 59. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
- 60. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:2.
- 61. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:4.
- 62. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:6.
- 63. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:8.
- 64. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:10.
- 65. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:12.
- 66. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:14.
- 67. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:16.
- 68. The method of claim 57, wherein the polynucleotide is further defined as comprising a region encoding an amino acid sequence as set forth in SEQ ID NO:18.
- 69. The method of claim 57, wherein the region has the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, or SEQ ID NO:19.

- 70. The method of claim 69, wherein the region has the sequence of SEQ ID NO:1.
- 71. The method of claim 69, wherein the region has the sequence of SEQ ID NO:3.
- 72. The method of claim 69, wherein the region has the sequence of SEQ ID NO:5.
- 73. The method of claim 69, wherein the region has the sequence of SEQ ID NO:7.
- 74. The method of claim 69, wherein the region has the sequence of SEQ ID NO:9.
- 75. The method of claim 69, wherein the region has the sequence of SEQ ID NO:11.
- 76. The method of claim 69, wherein the region has the sequence of SEQ ID NO:13.
- 77. The method of claim 69, wherein the region has the sequence of SEQ ID NO:15.
- 78. The method of claim 69, wherein the region has the sequence of SEQ ID NO:17.
- 79. The method of claim 69, wherein the region has the sequence of SEQ ID NO:19.
- 80. The method of claim 57, wherein the polynucleotide is comprised in a vector.
- 81. The method of claim 80, wherein the vector comprises a promoter.
- 82. The method of claim 80, wherein the vector is a viral vector.
- 83. The method of claim 80, wherein the vector is a retroviral vector.
- 84. The method of claim 80, wherein the vector is a plasmid.
- 85. The method of claim 57, wherein the host cell is further defined as a prokaryotic cell.
- 86. The method of claim 57, wherein the host cell is a eukaryotic cell.
- 87. The method of claim 86, wherein the host cell is a mammalian cell.
- 88. The method of claim 87, wherein the host cell is a human cell.